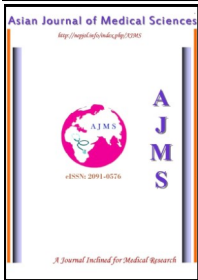


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The Protective Role of *Sphenocentrum Jollyanum* Leaf Extract on Beta Cells Morphology of Alloxan Treated Rabbits

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Abstract

Objective: To assess the protective effect of ethanol leaf extract of *Sphenocentrum jollyanum* (SJ) (Menispermaceae) on beta cells against alloxan treated rabbits.

Material & Methods: Rabbits of both sexes, divided into 6 groups (8 per group) received oral treatment as follows: groups A and B received Tween 80 (2 %) solution; group C received 10 mg/kg of glibenclamide; groups D, E and F received 50, 100 and 200 mg/kg of SJ. A week later (day 0), basal glycaemia was determined (72.4±3.5) followed by alloxan challenge at 170 mg/kg intravenously with the exception of group A. Blood was collected at days 0, 3, 5, 10 and 15 and analyzed. Inflammatory activity was evaluated using C - reactive protein (CRP) as biomarker. Superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (TBARS) and glutathione (GSH) were used to assess oxidative activity.

Results: At day 3, glycaemic increase of 157.2 ± 4.7, 153.2 ± 8.2 and 150.2 ± 9.5 (50, 100 and 200 mg/kg) was observed in the group that received SJ. Decrease however occurred from day 5 which continued to the last day of the experiment. On the contrary, progressive increase occurred in diabetic control. Tissue histology of the extract group showed no lesion. The CRP level which was slightly elevated in the extract group at day 3, decreased appreciably at day 15. Decrease in SOD and CAT activities which occurred at day 3 in SJ treated increased at day 15. Also, the elevated levels of hepatic GSH in extract treated demonstrated an enhanced biosynthesis of antioxidative enzymes.

Conclusion: SJ leaf effectively protected the beta cells against oxidative damage.

Key Words: beta cells; morphology; protection; *Sphenocentrum jollyanum*

1. Introduction

Diabetic mellitus (DM) is a chronic multifactorial disease that is characterized by hyperglycaemia.¹ The incidence of this disease has reached an alarming proportion constituting a major global health problem in the 21st century. Although a considerable inroad has been made to understand its pathophysiology, it is a disease that still defies effective control. DM has been identified as a clinical syndrome with the highest rate of prevalence and mortality worldwide.^{2,3} In DM, the persistent high level of blood glucose enhances the production of reactive oxygen species (ROS) from protein glycation and glucose autoxidation resulting in lipid peroxidation.^{4,5} Accordingly, there is sharp reduction in endogenous free radical scavenging mechanisms responsible for breaking down ROS activity.⁶

Sphenocentrum jollyanum (SJ) is a rain forest plant, a tiny shrub that belongs to the family of Menispermaceae (Pierre). It grows naturally along the west coast of sub-Saharan African countries where it has been traditionally used for the remedy of different diseases. The leaf is elliptical in shape and grows up to 20cm in length with the breadth of 5-12cm.⁷ SJ leaf has been shown to display a wide spectrum of biological and pharmacological activities. Its medicinal importance was first reported by Dalziel⁸ in which it was noted that the leaves decoctions were used as vermifuge. It is reputed for use in dressing wounds particularly chronic wounds, feverish conditions, cough as well as being an aphrodisiac.^{8,9} This part of the plant has been scientifically validated to possess significant anti-inflammatory, anti-angiogenic and analgesic properties. It has equally been found to be potent against polio type-2 virus¹⁰ while its antidiabetic effect

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has also been verified.¹¹

Since high level of ROS has been found to play a role in the pathogenesis of DM^{12,13}, anti-diabetic compound with anti-inflammatory and anti-oxidant properties will be a good prophylaxis against DM. This study was therefore, designed to investigate the protective, anti-inflammatory and anti oxidative role of SJ leaf on rabbits challenged with alloxan monohydrate.

2. Material and Methods

2.1. Plant material

The aerial part of SJ was obtained in the wild from a farm land located in Ijebu-Oru community, Ogun State, Nigeria. They were authenticated by a taxonomist, Dr. O. A. Ugboju, of the Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimen has been deposited in the herbarium (FHI/108203).

2.2. Preparation of plant ethanol extract

The leaves of SJ having been dried (35-37 °C) was subjected to size reduction to a coarse powder with an electric grinder. The powder (940g) was placed in a Soxhlet extractor and extracted with absolute ethanol in three cycles for about 48h. The extracted material was filtered with Whatman filter paper No. 4. The filtrate obtained was dried in vacuo at 30 °C. The yield about 37g was stored in an air tight bottle and kept in a refrigerator at 4 °C till it was needed.

2.3. Animals

Healthy adult rabbits of either sex weighing between 1.4-1.7 kg were obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria. Having certified their health conditions, they were kept in aluminum cages under natural light and dark cycle at the temperature of 26±5 °C in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. They were fed standard rabbit pellets from Livestock Feeds PLC, Lagos and water ad libitum. The use of the animals and the experimental protocol was in strict compliance with the standard guidelines on the use and care of experimental animals. The animals were weighed before the alloxan challenge (day 0) and on days; 3, 5, 10 and 15.

2.4. Prophylactic study

Rabbits randomly divided into 6 groups (8 in each), received oral treatment as follows: groups A and B received only distilled water; group C received 10 mg/kg body weight (bw) of glibenclamide; groups D, E and F

received 50, 100 and 200mg/kg bw of the extract respectively by gavages. A week later (day 0), basal glycaemia was determined after overnight fast followed by alloxan monohydrate challenge at a lethal dose of 170 mg/kg bw intravenously against 150 mg/kg¹⁴ used to induce hyperglycaemia in animal model. Blood was collected at days 0, 3, 5, 10 and 15 and analyzed by glucose oxidase method.

2.5. C-reactive protein (Latex) high sensitive assay

The evaluation of high sensitive C-reactive protein (hsCRP) was by turbidimetric analysis. Blood serum collected was centrifuged at 4000 rpm for 10 minutes. The turbidity that resulted from the reaction between serum and specific antibody for hsCRP was measured photometrically through the principle of particle-enhanced immunoturbidimetric assay.¹⁵

2.6. Oxidative activity assessment

The oxidative activity assessment was conducted in two batches, at day 3 and day 15. In the first batch, after overnight fast and blood collection, half of the animal population randomly selected from each group was sacrificed while the second batch was sacrificed at the end of the study. The animals were sacrificed under mild diethyl ether anaesthesia. The hepatic tissue harvested were homogenized and used for the assays.

2.7. Superoxide dismutase assay

Superoxide dismutase (SOD) was assayed utilizing the technique of Kakkar et al.¹⁶ A single unit of the enzyme was expressed as 50% inhibition of Nitroblue Tetrazolium (NBT) reduction/min/mg protein which was measured spectrophotometrically at 420nm.

2.8. Catalase assay

Catalase (CAT) was assayed colorimetrically at 620nm and expressed as μmoles of H₂O₂ consumed/min/mg protein.¹⁷ The hepatic tissue was homogenized in isotonic buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm for 10 minutes. The reaction mixture contained 1.0 ml of 0.01M (pH 7.0) phosphate buffers which was added to 0.1ml of tissue homogenate and 0.4 ml of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios).

2.9. Estimation of lipid peroxidation

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of

Niehaus and Samuelsson¹⁷ and expressed as nmol/ml. In brief, 0.1 ml of hepatic tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.3%, 0.25N HCL and 15% trichloroacetic acid) and placed in water bath for 10 min at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535nm.

2.10. Estimation of glutathione

The glutathione (GSH) level was determined by the method of Ellman.¹⁷ To the hepatic homogenate was added 10% trichloroacetic acid (TCA) and centrifuged. 10 ml of the supernatant was treated with 0.5 ml of Ellmans' reagent in 100 ml of 0.1% of sodium nitrate and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm.

2.11. Tissue histology

The pancreatic tissue was fixed in Bouin's fluid for four days before being embedded in paraffin wax. A section of each pancreatic tissue at 5 μ m was stained with aldehyde fuschsin which colours the beta cells purple. Each section was examined under light microscope at high power magnification for structural changes and photomicrographs were taken.

2.12. Phytochemical screening

Phytochemical screening of the extract for the presence of secondary metabolites was performed using the following reagents and chemicals: alkaloids with Mayer's and Dragendorffs reagents.^{18,19} Flavonoids with the use of Mg and HCl^{20,21}, tannins with 1% gelatin and 5% ferric chloride solution, and saponins with ability to produce suds.²¹ Liebermann- Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (19:1) was used to differentiate terpenoids and steroidal compound.¹⁸

2.13. Acute toxicity study

Mice randomly grouped (8 per group) received different doses (0.5, 1, 2, 4, 8 and 16 g/kg) of the extract administered by gavages after food was withdrawn for 24 hours. The animals were observed continuously for the first 4 hours and then for each hour for the next 12 hours, followed by 6 hourly intervals for the next 56 hours (72 hrs observations) to observe any death or changes in general behavior and other physiological activities.^{22, 23}

2.14. Statistical analysis

All values were expressed as mean \pm standard error of

mean and the statistical significance between treated and control groups were analyzed using the Student's t-test. $p < 0.05$ was considered significant. The difference of two means at 95 % and 99 % confidence levels were used for the analysis of the two sample sizes (days 3 and 15).

3. Results

Using the difference of two means, at 95 % and 99 % confidence levels, no significant changes were observed between the values obtained at days 3 and 15.

3.1. Variation of body weight

The body weight changes of the control and the groups that received extract /glibenclamide are shown in Fig-1. A reduction in body weight was observed in the animals at day 3 after alloxan challenge. From day 5, weight gain was recorded which showed progressive increase till the end of the experiment.

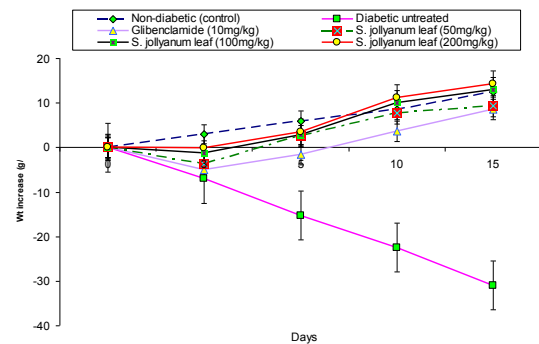


Figure-1: Weight increased in the animals

3.2. Prophylactic study

The antihyperglycaemic activity of SJ leaf is shown in fig. 2. Following alloxan challenge, the extract inhibited diabetic response in dose dependent manner. The maximum glucose levels of 157.2 \pm 3.5, 153.2 \pm 4.0, and 150.1 \pm 2.1 (50, 100 and 200 mg/kg respectively) were recorded at day 3 in the group that received SJ compared to the increase of 344.2 \pm 6.5 in the diabetic control group.

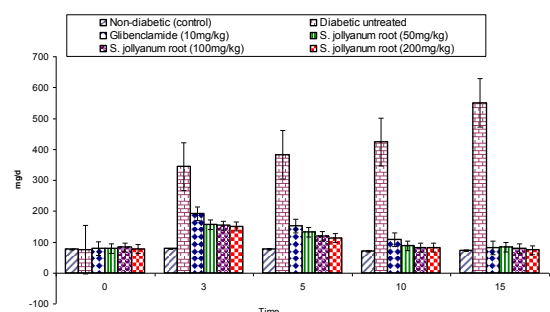


Figure-2: Prophylactic activity of SJ leaf. Values represent mean \pm SEM (n=8)

Table 1: Lipid level of rabbits treated with SJ leaf extract

	Dose (mg/kg)	Plasma lipid levels in control and treated animals ($\mu\text{mol}/\text{min}/\text{mg protein}/100\text{ml}$)							
		Day 3				Day 15			
		SOD	CAT	TBARS	GSH	SOD	CAT	TBARS	GSH
Normal		0.8 \pm 0.1	4.2 \pm 0.0	5.6 \pm 0.1	0.3 \pm 0.6	0.8 \pm 0.3	4.2 \pm 0.0	5.6 \pm 0.1	0.3 \pm 0.1
Diabetic		0.2 \pm 0.3	1.9 \pm 0.1	10.2 \pm 0.8	0.2 \pm 0.7	0.2 \pm 0.0	0.6 \pm 0.0	16.5 \pm 0.5	0.1 \pm 0.0
Diabetic + Glibenclamide	10	0.5 \pm 0.3	3.2 \pm 0.1	7.8 \pm 0.0	0.3 \pm 0.1	0.7 \pm 0.1	3.7 \pm 0.1	6.0 \pm 0.1	0.3 \pm 0.1
Diabetic + SJ	50	0.5 \pm 0.5	3.2 \pm 0.0	8.0 \pm 0.4	0.3 \pm 0.1	0.7 \pm 0.0	4.1 \pm 0.1	6.3 \pm 0.1	0.3 \pm 0.0
Diabetic + SJ	100	0.6 \pm 0.1	3.5 \pm 0.1	7.5 \pm 0.3	0.4 \pm 0.2	0.8 \pm 0.1	4.2 \pm 0.1	5.6 \pm 0.0	0.3 \pm 0.0
Diabetic + SJ	200	0.6 \pm 0.4	3.5 \pm 0.3	7.1 \pm 0.1	0.4 \pm 0.4	0.8 \pm 0.1	4.3 \pm 0.3	5.6 \pm 0.2	0.3 \pm 0.4

Table shows lipid concentration during 15 days of extract/glibenclamide administration or 10mg/kg distilled water (control). Values are Mean \pm SEM; n=6, *p<0.05 compared to control (Student's t-test).

However, at day 5, decrease occurred with percentage decrease of 16.5, 23.0 and 25.3 (50, 100 and 200 mg/kg respectively) while the animals that received glibenclamide recorded percentage decrease of 20.3. Decrease in blood glucose level continued in SJ/glibenclamide treated groups. At day 10, the blood glucose level of SJ treated at 200 mg/kg dose was comparable to normal control which also showed more effective decrease compared to the standard reference drug.

3.3. C-reactive protein activity

Increase in CRP concentration occurred following alloxan challenge. In the diabetic control (Fig. 3), the CRP level showed significant increase ($p < 0.05$) at day 3 that continued to the last day of the experiment. In SJ and glibenclamide groups, marginal increase in CRP levels occurred at day 3 that showed considerable decrease at day 15 with percentage decrease of 79.3, 81.5 and 81.8 (50, 100 and 200 mg/kg respectively) and 80.8 % for the glibenclamide group.

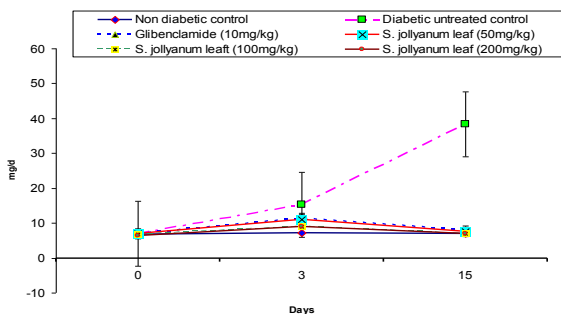


Figure-3: Activity of the leaf extracts on the C Reactive Proteins Values represent mean \pm SEM (n=8)

3.4. Effects on antioxidant profile

There was varied oxidative response resulting from alloxan challenge. Alloxan affected the homeostasis of SOD. The decrease in SOD level that was significant ($p < 0.05$) in diabetic control at day 3 while slight decrease occurred in extract and glibenclamide treated compared to the normal control. At day 15, SOD increased considerably to a level comparable to normal control in SJ and glibenclamide groups while the diabetic control group recorded further decrease in the SOD level.

CAT equally showed significant decrease in activity similar to SOD in diabetic control group while marginal decrease occurred in both the SJ and glibenclamide administered groups at day 3. At day 15, CAT activity rose appreciably to a level comparable to the normal control in the SJ and glibenclamide administered groups.

The hepatic TBARS evaluation showed increase in activity following alloxan challenge. In diabetic control, the lipid peroxidation activity showed progressive increase compared to normal while in SJ and glibenclamide groups, maximal increase occurred at day 3. At day 15, TBARS level decreased considerably in both the extract and glibenclamide groups.

Alloxan challenge led to significant decrease in hepatic GSH activity at day 3 in diabetic control compared to the normal. In SJ group, GSH exhibited increase in level at day 3 compared to the normal. Although slight decrease occurred at day 15, the activity was, however, comparably higher than the normal. Similarly, in glibenclamide group, GSH showed increase in activity.

3.5. Histopathology of pancreatic tissue

The aldehyde fuchsin stain demonstrated the

morphological features of beta cells. In the normal, the tissue morphology showed intact beta cells arrangement in situ that was devoid of deep nuclear staining. The photomicrograph of the extract administered group. No lesion while mild cellular lesion which led to partial shrinkage occurred in glibenclamide treated. The photomicrograph of diabetic control showed extensive necrotic changes forming a shrunken mass of amorphous eosinophilia with a halo around it (Fig-4).

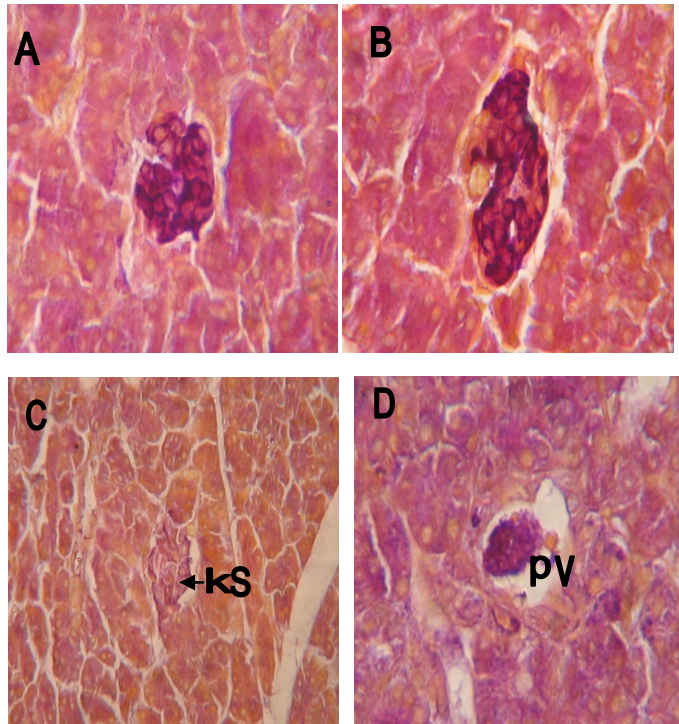


Fig. 4: (a-d), photomicrograph showing transverse section of pancreatic tissue of rat. Fig. 4a is a normal pancreatic tissue. Arrow shows beta cell in light purple. Fig. 4b shows pancreatic tissue of animal treated with leaf extract of RH with the beta cells appearing unaffected by alloxan challenge. Fig. 4c indicates pancreatic tissue of glibenclamide treated. Arrow (ks) shows partial shrinkage of islet mass resulting from necrotic changes on beta cells. Fig. 4d is a section of pancreatic tissue of untreated diabetic animal showing shrunken mass of islet cells with a halo (pv) around it. Mag. X 400.

3.6. Phytochemical screening

The active compounds found in the extract include; alkaloids, saponins, terpenoids, anthraquinones, flavonoids and tannins.

3.7. Acute toxicity test

There was 100 % mortality at the extract dose of 16g while no death occurred in the animals that received 4.0 g/kg bwt and less. The median acute toxicity (LD50) of the extract was determined to be 11.5 g /kg body weight.

4. Discussion

Alloxan which is known to cause free radical release

during its metabolism activates the development of alloxan-induced diabetes.²⁴ In this study, administration of SJ leaf extract at the doses of 50, 100 and 200 mg/kg respectively prior to and after alloxan monohydrate challenge at 170 mg/kg suppressed diabetic activity in rabbits. The precise mode of SJ activity is not known but since alloxan uptake is thought to be highly specific to islet cells²⁴, it is probable that SJ may have attenuated the selective uptake of the diabetogenic agent in the pancreatic islet. The tissue morphology showed no pathological changes and thus was confirmatory that the extract inhibited alloxan cytotoxic effect on pancreatic beta cells.

The body weight decrease observed at day 3 might be due to decrease in appetite following alloxan challenge. The progressive weight gain recorded from day 5 suggested that the extract apart from suppressing alloxan activity may have equally improved appetite to enhance eating.

Acute phase protein (C-reactive protein) comprise large heterogeneous group of proteins that are mainly synthesized in the liver. This inflammatory biomarker is usually known to increase in serum level during inflammatory process.²⁵ In this study, the extract exhibited anti-inflammatory activity by lowering the CRP level in a dose dependent manner when compared to the diabetic control. The observed initial rise in the level of CRP could have been due to acute phase activity caused by the inflammation-mediated action of alloxan.

The antioxidant enzymes, SOD and CAT are known for their vital role in reducing oxidative stress. SOD, a major intracellular antioxidant enzyme inactivates superoxide anion reducing it to hydrogen peroxide and oxygen. CAT on the other hand, working closely with SOD, converts H_2O_2 into H_2O and O_2 hence diminishes their toxic effect.²⁶ The result of this study showed that the extract led to an increase in hepatic SOD and CAT levels. This rise may appear as compensatory mechanism in response to an increase in oxidative stress.

Lipid peroxidation is one of the characteristic features that mediate tissue damage in diabetic condition.^{4, 27} The elevated level of peroxidation is known to affect drug metabolizing activity in diabetes.²⁸ The rise in TBARS observed at day 3 in extract and glibenclamide groups may be linked to acute phase activity caused by the lethal dose of alloxan. The decrease that occurred at day 15 suggested that the extract and glibenclamide

may have exerted antioxidant effect that mopped up ROS activity generated by the diabetogenic agent. SJ is rich in flavonoids and diterpenoids¹⁰ which are potent antioxidants²⁹ that scavenge free radicals and protect tissue from lipid peroxidation.

GSH which is a tripeptide normally present in large quantity intracellularly is perhaps the most important biomolecule that participate against chemically induced toxicity.³⁰ It is also known to protect the cellular system against the toxic effects of lipid peroxidation.³¹ The elevated level of hepatic GSH in the extract groups is indicative that the extract enhanced the biosynthesis of the antioxidative enzymes to protect the pancreatic beta cells against alloxan peroxidative activity. Similar account of plants showing protection on beta cells in alloxan induced animals has been reported.³²

5. Conclusion

This study demonstrated that ethanol leaf extract of SJ possesses significant anti-inflammatory and antioxidant activity that protected the beta cells against oxidative damage. The leaf of this medicinal plant might therefore serve as a useful therapy for reducing oxidative stress.

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6. References

1. Scoppola A, Montecchi FR, Mezinger G, Lala A. Urinary mevalonate excretion rate in type 2 diabetes: role of metabolic control. *Atherosclerosis*, 2001; 156: 357. [http://dx.doi.org/10.1016/S0021-9150\(00\)00660-2](http://dx.doi.org/10.1016/S0021-9150(00)00660-2)
2. Harris M, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance on US adults. The third nation and nutrition examination survey. *Diabetes Care*, 1998; 21: 518-26. <http://dx.doi.org/10.2337/diacare.21.4.518> PMID:9571335
3. Barcelo A and Rajpathak S. Incidence and prevalence of diabetes mellitus in the Americas. *Pan American Journal of Public Health*, 2001; 10: 300-8. <http://dx.doi.org/10.1590/S1020-49892001001100002>
4. Feillet-Coudray C, Rock E, Coudray C, Grzelkowska K, Azais-Braesco V, Dardevet D, et al. Lipid peroxidation and antioxidant status in experimental diabetes. *Clin. Chem Acta*, 1999; 284: 31-4. [http://dx.doi.org/10.1016/S0009-8981\(99\)00046-7](http://dx.doi.org/10.1016/S0009-8981(99)00046-7)
5. Latha M and Pari L. Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some poly pathway enzymes in experimental rat diabetes. *Braz J Med Biol Res.*, 2004; 37: 577-86. <http://dx.doi.org/10.1590/S0100-879X2004000400015> PMID:15064821
6. Oberley LW. Free radicals and diabetes. *Free Radic Biol Med.*, 1988; 5: 113-24. [http://dx.doi.org/10.1016/0891-5849\(88\)90036-6](http://dx.doi.org/10.1016/0891-5849(88)90036-6)
7. Nia R, Paperz DH, Essien EE, lyadi KC, Bassey ALL, Antai AB, et al. Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum jollyanum* Pierre. *African J of Biomed Res.*, 2004; 7:129-32.
8. Dalziel JM. In: *The useful plants of West Africa*. Crown agents for overseas governments and administration, London, 1955. pp 15.
9. Iwu MM. *Handbook of African medicinal plants*. CRC Press Inc. 1993, pp 239.
10. Moody JO, Roberts VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). *J Ethnopharmacol.*, 2006; 104: 87-91. <http://dx.doi.org/10.1016/j.jep.2005.08.051> PMID:16236477
11. Mbaka GO, Adeyemi OO, Noronha CC, Anunobi CC, Okanlawon AO. Anti-hyperglycaemic effects of ethanol leaf extract of *Sphenocentrum jollyanum* in normal and alloxan-induced diabetic rabbits. *Global Journal Pharmacol.*, 2008; 2 (3): 46-51.
12. Hartnett E, Straton R, Browne R, Rosner B, Lanham R, Armstrong D. Serum markers of oxidative stress and severity of diabetic retinopathy. *Diabetes Care*. 2000; 23: 234-40. <http://dx.doi.org/10.2337/diacare.23.2.234> PMID:10868837
13. Punitha ISR, Rajendran K, Shirwaikar A, Shirwaikar A. Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. *eCAM*. 2005; 10: 1-7.

14. Abdel-hassan IS, Abdel-Barry JA, Mohammeda ST. The hypoglycaemic and antihyperglycaemic effect of Citrullus cocoythis fruit aqueous extract in normal and alloxan diabetic rabbits. *J. Ethnopharmacol.*, 2000; 71: 325-30. [http://dx.doi.org/10.1016/S0378-8741\(99\)00215-9](http://dx.doi.org/10.1016/S0378-8741(99)00215-9)
15. Kushner I, Gewurz H, Benson MD. C-reactive protein and the acute-phase response. *J Lab Clin Med.*, 1981; 97: 739-49. PMID:7229508
16. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase (SOD). *Ind J Biochem Biophys.*, 1984; 21: 130-2. PMID:6490072
17. Rukkumani R, Aruna K, Varma PS, Rajasekaran KN, Menon VP. Comparative effects of curcumin and analog of curcumin on alcohol and PUFA induced oxidative stress. *J Pharm Sci.* 2004; 7(2): 274-83.
18. Farnsworth RN. Review on Biological and Phytochemical screening of plants. *J. Pharm. Sci.* 1966; 55: 225-276. <http://dx.doi.org/10.1002/jps.2600550302> PMID:5335471
19. Harborne JB. *Methods of extraction and isolation in: Phytochemical methods.* London: Chapman and Hall, 1998, pp: 60-6.
20. Silva LG, Lee IS, Kinghorn DA. Special problems with the extraction of plants. In: *Methods in biotechnology natural product isolation.* Cannell JPR (ed) Humana, Press Inc., Totowa, New Jersey, USA., 1993; pp: 329-363.
21. Houghton PJ and Raman A. *Laboratory handbook for fractionation of natural extracts.* Chapman and Hall, London, 1998; pp: 199. <http://dx.doi.org/10.1007/978-1-4615-5809-5>
22. Shah AMA, Garg SK, Garg KM, Subacute toxicity studies on Pendimethalin in rats. *Indian J Pharmacol.*, 1997; 29:322-4.
23. Berger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC, Soares dos Santos, AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasiliensis*) (Asteraceae) in mice. *Journal Pharm Sci.* 2005; 8 (2): 370-3.
24. Ho E, Chen G, Bray TM. Supplementation of N-acetylcysteine inhibits NFkB activation and protects against alloxan-induced diabetes in CD-1 mice. *FASEB J.* 1999; 13: 1845-54. PMID:10506589
25. Asgari SA, Mokhtari G, Falahatkar S, Mansour-Ghanaei M, Roshani A, Zare A, et al., Diagnostic accuracy of C-reactive protein and erythrocyte sedimentation rate in patients with acute scrotum. *Urology Journal* 2006; 3 (2): 104-6. PMID:17590844
26. Baynes JW. Reactive oxygen in the aetiology and complications of diabetes. In: Ioannides C, Fatt P. R, editors. *Drug, diet and disease vol 2: mechanistic approach to diabetes.* Hertfordshire: Ellis Horwood ltd, 1995; pp 203-31.
27. Bavarva JH and Narasimhacharya AVRL. Antihyperglycaemic and hypolipidemic effects of *Costus speciosus* in alloxan induced diabetic rats. *Phytother. Res.* 2008; 22: 620-6. <http://dx.doi.org/10.1002/ptr.2302> PMID:18444247
28. Levin W, Lu AY, Jacobson M, Kuntzman R, Poyer JL; McCay PB. Lipid peroxidation and the degradation of cytochrome P-450 heme. *Arch Biochem Biophys.* 1973; 158: 842-52. [http://dx.doi.org/10.1016/0003-9861\(73\)90580-8](http://dx.doi.org/10.1016/0003-9861(73)90580-8)
29. Hayashi T, Kawaski M, Miwa Y, Taga T, Morita N. Antiviral agents of plant origin III. Scopadulin, a novel tetracyclic diterpene from *Scoparia dulcis* L. *Chemical and Pharmaceutical Bulletin.* 1990; 38: 945-947.
30. Hye KK, Mi JK, Eun SL, Dong-Hoon S, Improvement of diabetic complication by *hydrangea dulci folium* in streptozotocin-induced diabetic rats. *Biol. Pharm Bull.* 2009; 32 (1): 153-6. <http://dx.doi.org/10.1248/bpb.32.153>
31. Nicotera P and Orrenius S. Role of thiols in protection against biological reactive intermediates. *Adv Exp Med Biol.*, 1986; 197: 351-8.
32. Lee J, Park J, Kim J, Park B, Rho H. Protective effect of amomi semen extract on alloxan induced pancreatic beta cell damage. *Phytotherapy Res.*, 2008; 22: 8686-90. <http://dx.doi.org/10.1002/ptr.2271> PMID:17724760